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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <b>(21) International Application Number:</b> PCT/NL98/00720<br><b>(22) International Filing Date:</b> 18 December 1998 (18.12.98)<br><b>(30) Priority Data:</b><br>97203991.1 18 December 1997 (18.12.97) EP<br><b>(71) Applicant (for all designated States except US):</b> INSTITUUT VOOR AGROTECHNOLOGISCH ONDERZOEK (ATO-DLO) [NL/NL]; P.O. Box 17, NL-6700 AA Wageningen (NL).<br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only):</b> JONGBOOM, Remigius, Oene, Jules [NL/NL]; Damstraat 28, NL-6671 AE Zetten (NL). STUUT, Peter, Iwan [NL/NL]; Stationsstraat 54, NL-6701 AM Wageningen (NL). HOVENS, Irene, Antoinette, Petra [NL/NL]; Groenstraat 37, NL-5913 CB Venlo (NL).<br><b>(74) Agent:</b> DE BRUIJN, Leendert, C.; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL). |           | <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>Without international search report and to be republished upon receipt of that report.</i> |
| <b>(54) Title:</b> COMPOSITION FOR THE CONTROLLED RELEASE OF ACTIVE COMPOUNDS<br><b>(57) Abstract</b><br><p>The invention provides a composition for the controlled release of one or more biologically active substances encapsulated in a degradable biopolymer matrix, consisting of a thermoplastic and/or partly crystalline inulin. A plasticiser such as glycerol, and an emulsifier may be present. The active substance is e.g. a drug, a biocide, a fertiliser, a flavour, a protein or a microorganism.</p>   |           |  |

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## COMPOSITION FOR THE CONTROLLED RELEASE OF ACTIVE COMPOUNDS

The present invention relates to a composition for the controlled release of one or more active compounds, especially biologically active compounds, from a degradable matrix material.

5        Such a composition is known from WO 94/01092. This known composition contains the active substance, which may be a protein or other high molecular weight compound, in a matrix consisting of a helical, crystalline straight-chain  $\alpha$ -glucan such as amylopectin or crystalline amylose. A similar composition is known from WO 94/01091 wherein the matrix further contains a glucan-degrading enzyme such as an  
10         $\alpha$ -amylase.

It was found that a composition for the delayed release of an active substance can be produced by using a thermoplastic and/or crystalline fructan as a matrix material for said composition, together with one or more active substances. The composition of the invention and the process of producing it are defined in the appending claims.

15        The fructan to be used is inulin (poly- $\beta$ -2,1-fructose terminated at the reducing end with 1-glucosyl). The inulin can have a varying chain length, from 3 up to as high as 3,000. Preferred chain length are from 3 to 70, particularly from 5 to 60, especially from about 7 to about 35. The inulin may be native inulin, or it may be physically and/or chemically modified. Such modifications include fractions having a lower or higher  
20        average chain length, hydrolysates, enzymatically shortened or lengthened treated inulins, partially oxidised inulins and the like. Inulins have the advantage of having a varying degree of crystallinity, depending on the processing of the inulin and on the water content. The degree of crystallinity can control the release pattern of the matrix material in an aqueous medium, as a higher crystallinity results in slower dissolution and hence  
25        in slower release. The crystallinity can be assayed by means of X-ray analysis. Preferably, the inulin is partly crystalline, i.e. it has a structure between that of  $\alpha$ -inulin (anhydrous, amorphous) and of  $\beta$ -inulin (hemihydrate or monohydrate, crystalline). Preferably, the inulin contains 0.1-1.2 mol, in particular 0.3-1 mol, of crystal water per  
30        mol of anhydrofructose. The crystallinity and thus the release rate of encapsulated materials can also be influenced by using an inulin of varying polydispersity or by admixing other biopolymers, such as starch or hydrolysates or derivatives thereof,

cellulose derivatives or protein hydrolysates in amounts of e.g. 0-40 wt.%, especially 1-25 wt.% with respect to the inulin weight. Another factor that can be used to influence the release rate is the chain length of the inulin.

Active substances to be incorporated in the controlled-release composition include any substance which is introduced into an environment for performing a desired function. Examples include biologically active substances such as drugs, antigens, diagnostic agents, biocides, repellents and attractants (pheromones), flavours, fragrances, fertilisers, and other active substances such as colorants. The active substances can be relative simple inorganic (metals, metal compounds) or organic molecules, but also macromolecules, including carbohydrates, proteins (enzymes, antibodies, toxins), nucleotides, and biological systems including cells and microorganisms (bacteria, viruses).

Plasticisers may be used to enhance the thermoplastic nature of the biopolymer. Plasticisers to be used include water, glycols and other polyols, partial esters thereof, citric acid and urea. Especially preferred is glycerol. Plasticisers other than water may be used at a level of 0-50 wt.% with respect to the biopolymer, especially 1-30 wt.%.

The compositions may further contain an emulsifier and/or a flow regulator, such as lipids, phospholipids, such as lecithin, glycerol monoesters, higher alkyl ammonium salts, and the like. These agents are commercially available, under the brand names Tween, Span, etc. These emulsifiers may be present at a level of from 0, especially from 0.1 to 15 wt.% with respect to the inulin.

The process for producing the controlled-release compositions comprises mixing the inulin matrix material with the optional plasticiser and emulsifier and the active substance, and subjecting the mixture to a thermomechanical treatment. The thermomechanical treatment may comprise extrusion, rolling, kneading, injection-moulding, pelleting and similar treatments. This thermoplastic processing can be carried out at temperatures of 30-120°C, preferably using a plasticiser and/or a flow regulator. As an alternative, the active substance may be added after the thermomechanical treatment.

The composition of the invention can be used to release the active substance(s) in a controlled manner. The release can be triggered by the presence of water, acid and/or heat. As indicated above, the release rate can be adjusted by adjusting the composition and the conditions of the thermomechanical treatment. In particular, the

release rate can be adjusted by means of the average chain length (degree of polymerisation, DP) of the inulin. Inulins having a relatively low DP of, say, 3-10, will result in compositions with short release rate, whereas compositions with inulins having a relatively high DP, e.g. 15-60, as a matrix material will have slower rates. The release can further be controlled by means of the crystallinity (see above), the particle size, and the plasticiser content. Also, the affinity of the encapsulated material with the matrix determines the release, and the rate can thus be adapted by slightly modifying the matrix material, e.g. by increasing hydrophobicity (acylation, alkylation). The composition can be used in medicine, i.e. as a medicament to be introduced into the gastro-intestinal tract of a mammal. It can also be used in horticulture and agriculture, where the composition can be applied to the soil or onto plants or be atomised in the air.

## EXAMPLES

### *Materials*

Inulin used in the examples was commercially available Frutafit® from Suikerunie (NL). Glycerol was supplied by Chemproha BV (NL) and lecithin was supplied by Lucas Meyer (NL). Tween-60 and Span were obtained from Boom NV, Meppel, NL. The pheromone Muscalure and liquid synthetic orange (IFF BV, 15918261) and apple flavour (IFF BV, 15021196) were used as active substances to be encapsulated.

### **Example 1**

Eighty g of inulin (moisture content 5% w/w), 0-4.6 g of emulsifier (lecithin: L; tween 60: T; span 65: S), 0-11.5 g of glycerol (G) and 0-13 g pheromone (M) were mixed at room temperature to a homogeneous premix. Seventy five g of this premix was fed into a Haake Rheomix 600 kneader. The kneader had three zones, that could be heated separately. The middle zone was cooled by air. The premix was fed through a loading trap at the top. The kneader was operated for 10 min. at a screw rotation speed of 80 rpm. The kneading temperature was 120°C. The kneaded sample was then stored on an aluminium tray in a climate cell at 20°C and 60% relative humidity. The retention of the samples was determined by means of DSC (Differential Scanning Calorimetry) and the pheromone level was determined from the melt enthalpy of the pheromone in the sample. The results are summarised in table 1.

**Table 1**  
Kneading of inulin matrix containing pheromone

| exp | plas % <sup>1</sup> | emul % <sup>2</sup> | pher % <sup>3</sup> | torque (Nm) | retention <sup>5</sup> |
|-----|---------------------|---------------------|---------------------|-------------|------------------------|
| 1   | 0                   | 6 T                 | 5 M                 | 2.4         | 0.89                   |
| 2   | 0                   | 5 T                 | 10 M                | 1.4         | 0.90                   |
| 3   | 0                   | 5 T                 | 15 M                | 0.8         | 0.83                   |
| 4   | 0                   | 6 T                 | 20 M                | 0.7         | 0.70                   |
| 5   | 5 G                 | 5 T                 | 10 M                | 0.3         | 0.86                   |
| 6   | 10 G                | 10 T                | 10 M                | 0           | 0.53                   |
| 7   | 0                   | 5 S                 | 10 M                | 0           | 0.69                   |
| 8   | 15 G                | 3 L                 | 15 M                | 0.5         | 0.88                   |
| 9   | 15 G                | 3 L                 | 17 M <sup>4</sup>   |             | 0.19                   |

<sup>1</sup> amount (% w/w to inulin) of plasticiser (G = glycerol)

<sup>2</sup> amount (% w/w to inulin) of emulsifier (T = Tween 60; S = Span 65; L = Lecithin)

<sup>3</sup> amount (% w/w to inulin) of pheromone (M = Muscalure)

<sup>4</sup> pheromone was added to inulin melt

<sup>5</sup> retention of encapsulated substance expressed as the ratio of the amount in the sample after kneading and the amount before kneading

### Example 2

Inulin (moisture content 5% w/w) and 10 % (w/w to dry inulin) of Capsul-E emulsifier (C) were mixed with a Bear Varimixer for 10 minutes. The premix was fed into the extruder using a K-Tron K2M T85 volumetric feeder. The extruder was a Werner & Pfeiderer ZSK25 extruder, which is a self-wiping co-rotating twin screw extruder with a length of 34\*D (D = screw diameter). The extruder had 7 zones with the following temperature profile (°C): 50-80-120-120-100-80-80. Glycerol (G) was pumped into the first zone. Orange (O) or apple (A) flavour was introduced into the fifth zone (at 20.8\*D) using a pressure-independent volumetric pump. The inulin/emulsifier throughput was 7.1 kg/h. The flavour throughput was 0.3 and 0.6 kg/h and the glycerol throughput was 0.6 and 1.1 kg/h. The results are summarised in table 2. The flow rates (throughputs) are given in g/min.

**Table 2**  
Extrusion of inulin matrix containing flavour

| exp | flow inulin<br>+ emulsifier | flow<br>glycerol | flow<br>flavour | rotation<br>rate (rpm) | retention<br>(%) |
|-----|-----------------------------|------------------|-----------------|------------------------|------------------|
| 10  | 126                         | 10.7             | 9.7 O           | 300                    | 76               |
| 11  | 121                         | 10.7             | 9.7 A           | 300                    | 51               |

### Example 3

Eighty grams of inulin (moisture content 5%), 15.2 grams of glycerol (G) and 0–15.2 grams of native potato starch (PN) were mixed at room temperature to a homogeneous premix. 75 grams of this premix was fed into a Haake Rheomix kneader with roller rotors. This kneader had three zones that could be separately heated. One zone could be cooled by air. Into the kneader chamber a thermocouple was inserted by which the melt temperature was measured. At the top a loading trap was present by which premix was fed into the kneader. The kneader was operated for 10 minutes at a screw rotation speed of 80 rpm. For the different samples the kneading temperature was varied between 90 and 150°C. After kneading the samples were stored for one day at 20°C and 30% relative humidity.

The crystallinities of the samples were determined by X-ray diffraction. The samples were ground cryogenically and sieved to obtain a fine powder. The powdered samples were placed in sample holders and a smooth surface was created. The sample holders were placed in a Philips PC-APD PW 3710 mpd X-ray diffractometer supplied with a CuK $\alpha$  emitter operated at 50mA and 40kV. The samples were scanned at an angle of 5° to 40° (2 $\theta$ ) with a scanspeed of about 1.2° per minute. The scattered radiation was detected using proportional detection. The crystallinities of the inulin samples, processed at different temperatures and at different compositions are mentioned in figures 1 and 2. Fig. 1 depicts the crystallinity of inulin samples processed at 120°C with 20% glycerol (w/w to dry inulin) and increasing (0–10–20%) native potato starch content. Fig. 2 depicts the crystallinity of inulin samples processed at different temperatures (90–120–135–150°C) with 20% glycerol (w/w to dry inulin).



## CLAIMS

1. Composition for the controlled release of one or more biologically active substances encapsulated in a degradable biopolymer matrix, *characterised* in that the biopolymer comprises a thermoplastic and/or partly crystalline inulin.
2. Composition according to claim 1, wherein the inulin has between 0.1 and 1.0 mol of crystal water per mol of anhydrofructose.
3. Composition according to claim 1 or 2, wherein the inulin has an average chain length of 3 to 60 anhydrofructose units.
4. Composition according to any one of claims 1-3, wherein the biopolymer matrix contains 3-30 wt.% of a plasticiser with respect to the weight of the biopolymer.
5. Composition according to any one of claims 1-4, wherein the biopolymer matrix contains 1-15 wt.% of an emulsifier with respect to the weight of the biopolymer.
6. Composition according to any one of claims 1-5, wherein the active substance is encapsulated at a level of 0.001 to 80 % by weight, preferably 0.01-15 % by weight, with respect to the weight of the inulin matrix.
7. Composition according to any one of claims 1-6, wherein the active substance is a drug, a biocide, a fertiliser, a flavour, a protein or a microorganism.
8. Composition according to any one of claims 1-7, wherein the biopolymer further comprises 0-40 wt.% of starch, starch hydrolysates, starch derivatives, cellulose derivatives and/or protein hydrolysates.
9. Process of producing a controlled release composition according to any one of the preceding claims, *characterised* in that inulin, optionally mixed with a plasticiser and/or an emulsifier, is shaped to produce thermoplastic and/or partly crystalline inulin, and one or more biologically active substances are added to the inulin before or after shaping.
10. Process according to claim 9, wherein the shaping step is performed by extrusion or by kneading.

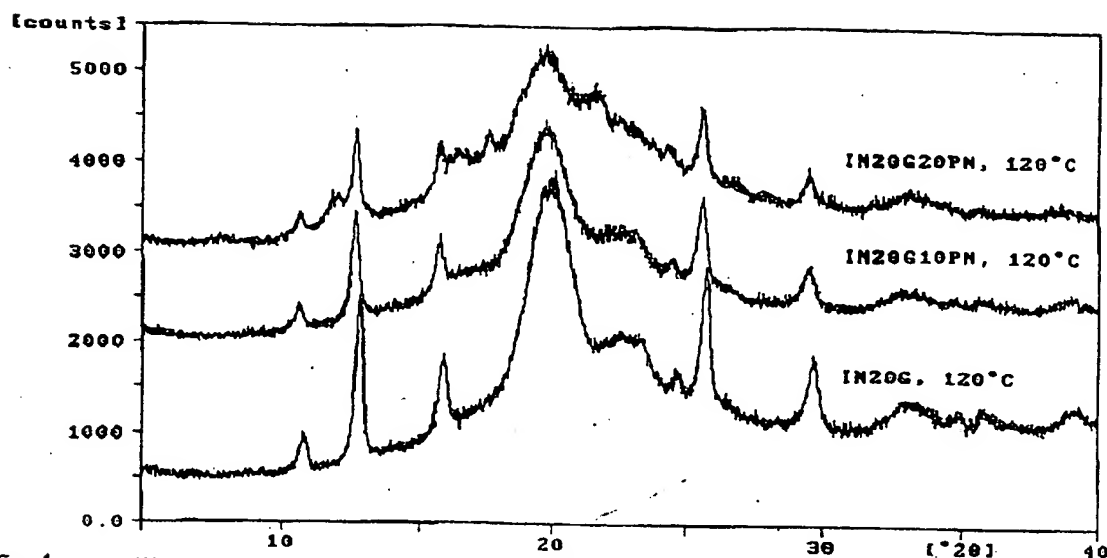


fig. 1: crystallinity inulin samples, processed at 120°C with 20% glycerol (w/w to dry inulin) and increasing native potato starch content

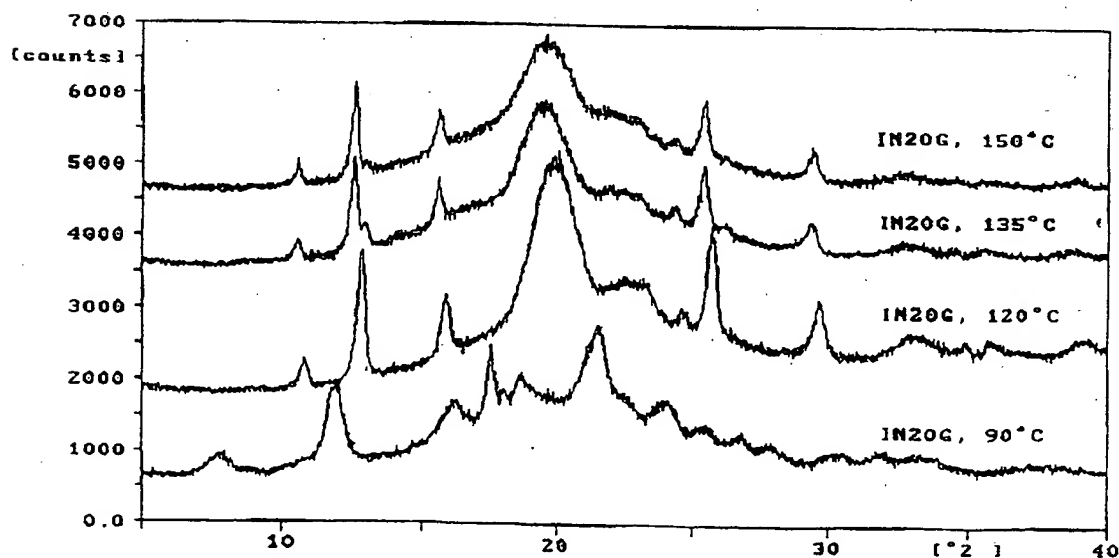


fig. 2: crystallinity inulin samples with 20% glycerol (w/w to dry inulin), processed at different temperatures